EFFECT OF VARYING DIETARY VITAMIN A SUPPLEMENTATION LEVELS IN COMBINATION WITH *ADH1C* GENOTYPE ON INTRAMUSCULAR FAT DEPOSITION IN FINISHING BEEF STEERS

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ABSTRACT

Previously, *ADH1Cc.-64T>C* was shown to have an association with intramuscular fat (IMF) in the *longissimus thoracis* (LT) muscle when vitamin A was limited in finishing rations of beef steers. The purpose of the current study was to determine the optimum vitamin A supplementation level, in combination with *ADH1C* genotype, to increase IMF of the LT muscle. Forty-five TT, 45 CT and 27 CC cross-bred steers, black in colour, were backgrounded on a commercial ration containing 3360 IU vitamin A/kg DM. During finishing the steers were randomly assigned to one of three vitamin A treatments at 25, 50 and 75% of the NRC recommendation of 2200 IU/kg DM. Treatments were administered via an oral bolus. Carcass quality was evaluated and a sample from the LT muscle was collected for analysis of IMF. A treatment x genotype interaction (P=0.04) was observed for IMF; TT steers on the 75% treatment had higher IMF relative to CT and CC steers on the same treatment. Intramuscular fat was also higher for TT steers on the 75% treatment in comparison to TT steers on the 25% treatment. Eighty-four percent of the steers graded Canada AAA. Western blot analysis showed that TT steers had higher (P=0.02) ADH1C levels in hepatic tissue. Previously, TT steers had increased IMF when fed limited vitamin A. In the current study the lack of variation between treatments and genotypes at the lower vitamin A treatment levels was likely due to the majority of the steers grading Canada AAA (USDA Choice). However, the western blot data supports that TT steers are expected to have higher IMF deposition, due to an increase production of ADH1C.

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LIST OF ABBREVIATIONS

%	Percent
µg	Micrograms
ADF	Acid Detergent Fiber
ADG	Average daily gain
ADH1C	Alcohol dehydrogenase 1C
BW	Body weight
C/EBP	CAAT/enhancer binding protein
CWT	Hundredweight or centum weight
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EE	Ether extract
IMF	Intramuscular fat
IU	International unit
NDF	Neutral detergent fiber
PCR	Polymerase chain reaction
RA	Retinoic Acid
RAL	Retinaldehyde
RALDH1	Retinaldehyde dehyrogenase
RAR	Retinoic acid receptor
RARE	Retinoic acid response element
RBP4	Retinol binding protein 4
REA	Rib-eye area
RFLP	Restriction fragment length polymorphism

ROL Retinol

SAS Statistical analysis system

SEM Standard error of the mean

SNP Single nucleotide polymorphism

TRT Treatment

USDA United States Department of Agriculture

1. INTRODUCTION

The beef cattle industry today is largely driven by consumer preference and demand. Marbling (intramuscular fat) is considered one of the major factors influencing the consumer's perception of meat quality. It is also one of the leading factors determining the economics of the feedlot industry. Cattle are marketed based either on live weight or dressed weight. In both cases, the quality grade given to each carcass is important. In some markets, particularly in the United States and in grid marketing programs in Canada, a premium is received for producing carcasses with consistently high marbling grades (DiCostanzo and Dahlen 2000).

One method of improving consistency in carcass quality is through marker assisted management (MAM), which involves managing cattle to improve efficiency based on the genotype of the animal. The leptin gene single nucleotide polymorphism (SNP) *LEP c.73C>T* is a current example, where genetic selection of animals that are TT at this SNP achieve a higher degree of marbling in the final carcass earlier than CC animals (Buchanan *et al.* 2007). Selecting and managing cattle based on the individual animals genotype can significantly increase the profit for producers.

Previous nutritional studies have found that restriction of vitamin A in the diet of feedlot cattle improves marbling scores (Oka *et al.* 1998; Gorocica-Buenfil *et al.* 2007a; Gibb *et al.* 2011; Pickworth *et al.* 2012). These studies reported no negative impact on average daily gain (ADG) or feed:gain ratio (F:G). The positive impact of vitamin A restriction on intramuscular fat deposition can be partially explained through nutrigenomics, whereby a bioactive component of food regulates gene expression. The vitamin A metbolites, retinaldehyde (RAL) and retinoic acid (RA) have the potential to regulate adipogenesis through interaction with nuclear receptor proteins. Ward *et al.* (2012) discovered a novel SNP in the gene coding for the enzyme ADH1C, which catalyzes the conversion of retinol (ROL) to RAL. The researchers conducted a nutrigenetic investigation to evaluate a possible link between this SNP and vitamin A supplementation level. They reported

that animals TT at the SNP had 23% higher IMF relative to CC steers when no vitamin A was supplemented. These results indicate the potential use of this SNP in feedlot marker-assisted management programs. However, complete restriction of vitamin A in the ration is impractical due to possible health ramifications associated with clinical vitamin A deficiency (NRC 1996).

The objective of the current research was to determine the optimum vitamin A supplementation level, in combination with *ADH1C* genotype, for backgrounding and finishing beef cattle diets in order to achieve conistently high quality grades.

2. LITERATURE REVIEW

2.1. Livestock Nutritional Research

Animal nutrition research has historically focused on examining specific nutrient toxicities and deficiencies, with the objective of determining optimal inclusion levels for maximum performance and production. However, new research into diet-gene interactions has begun to change the face of nutritional research (Mutch *et al*. 2005) through the development of two new fields: nutrigenomics (nutritional genomics) and nutrigenetics (nutritional genetics; Figure 2.1). Both of these fields allow for the better understanding of the interactions revolving around biological components of feed, genetic polymorphisms and the complex relationship with an individual's entire biological system (Mutch *et al*. 2005; Kaput and Rodriguez 2004).

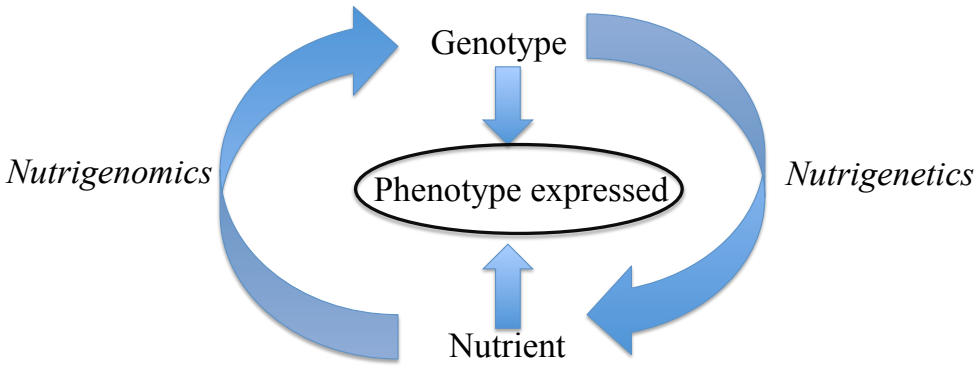

Figure 2.1. Schematic showing the interaction of genes and nutrients on phenotypic responses and the introduction of nutrigenetics and nutrigenomics in this complex. Modified from Farhud *et al*. 2010.

2.1.1. Nutrigenomics

Nutrigenomics attempts to use genome wide tools to explore the effects of nutrition on gene expression, function and regulation (Mutch *et al*. 2005). Techniques such as proteomics, metabolomics and microarrays (Kaput and Rodriguez 2004; Masotti *et al*. 2010), are used to combine information from genetics, nutrition, physiology, molecular biology and bioinformatics (Kaput and Rodriguez 2004; Kibbe 2006). The main objective of nutrigenomics in humans is to prevent the onset of disease and maintain health through dietary intervention (Kaput and Rodriguez 2004).

2.1.2. Nutrigenetics

Nutrigenetics is a branch of nutrigenomics that attempts to understand how genetic variants influence an individual's phenotypic response to a specific nutrient (Marti *et al*. 2010; Mutch *et al*. 2005). This field evaluates polymorphisms in genes from a pathway where nutrigenomics is occurring, which could lead to a unique management option for the beef cattle industry.

2.1.3. Nutrient-Gene Interactions

The beef cattle diet is a complex mixture of natural substances or nutrients that are or have the potential to be biologically active. These nutrients provide the fuel for normal body functions. In order for utilization to happen, a series of catabolic reactions within the body must occur. These processes require enzymes, which are proteins. Proteins are produced when a specific gene coding for that protein is expressed.

The integrity of a gene can be influenced by metabolic stimuli, whether internal or external, such as hormones or nutrients, respectively (Siddique *et al*. 2009; Kore *et al*. 2008). Nutrients are the major external stimuli influencing gene expression (Siddique *et al*. 2009), and can act directly or indirectly.

2.1.3.1. Direct

Nutrients directly impact gene expression largely through interaction with transcription factors, which control the flow of information from DNA through binding to specific regions of the DNA sequence. For example, the vitamin A derivatives (retinoic acid and retinaldehyde) interact with retinoid receptors (retinoid X receptor and retinoic acid receptor). These form homo- or heterodimers that interact with retinoic acid response element (RARE), which is located in the promoter region of the gene (Desvergne 2007; Repa *et al*. 1993; Heyman *et al*. 1992). When these interactions occur, gene expression can be up or down regulated. Nutrients such as Vitamin D interacting with vitamin D receptor, vitamin C with gene expression in lymphocytes (Wang *et al*. 2007), fatty acids with peroxisome proliferator-activated receptors (PPAR; Phillips *et al*. 2010; Hsu *et al*. 2006) or zinc deficiency influencing changes in gene expression for cholecystokinin and ubiquinone oxidoreductase (Blanchard and Cousins 2000) are other examples where nutrients impact gene expression. Vitamin A derivatives also have the potential to interact with PPAR proteins (Desvergne 2007; Ziouzenkova *et al*. 2007).

Genotypic variation, such as that represented by a single nucleotide polymorphism (SNPs), offers a potential for alteration in the nutrient-gene interaction complex. SNPs are a single nucleotide difference between individuals at the same locality (Lewin 2008). These alterations in the genetic code can result in truncated proteins, impaired protein function or in some cases have no influence on the protein product (Pey *et al*. 2007; Kimchi-Sarfaty *et al*. 2007). These changes, or lack thereof, result in alterations in promoter activity, mRNA conformation and subcellular protein location. Transcription factors rely on the nucleotide sequence of response elements to regulate gene expression. Therefore, when the nucleotide sequence is altered, the impact of nutrients on gene expression may also be altered.

2.1.3.2. Indirect

When there are no changes in the DNA sequence, nutrients can influence gene expression in an epigenetic manner. This is largely through DNA methylation pathways, which is a major epigenetic regulatory mechanism. It

is involved in a variety of cellular processes such as development, X-inactivation, chromosome stability and imprinting. A deficiency in folic acid, a dietary factor involved in one-carbon metabolism, results in a decrease in DNA methylation, and has been linked to anemia and cancer (Crider *et al*. 2012).

2.1.4. Applications of Nutrigenetics

In human research, nutrigenetics has been applied for several decades towards personalization of diets to achieve maximum health on an individual and genetic subgroup level (*reviewed* by Phillips 2013). This has influenced new advances in atherosclerosis research and prevention (Merched and Chan 2013) as well as prevention of various metabolic diseases including cardiovascular disease, insulin resistance and diabetes (*reviewed* by Phillips 2013). The knowledge of the impacts of nutrition on human health and disease prevention suggests that nutrigenetics could be applied to health and disease management of livestock, as well as managed feeding for specific phenotypic responses.

2.2. Marker-Assisted Management

Marker Assisted Management (MAM) is a practice that involves selecting and managing cattle to improve efficiency based on the genotype of the animal. Both genetic and nutritional background can have a major impact on the growth potential of beef cattle (Bruns *et al*. 2005; Platter *et al*. 2003).

Genetic variation (i.e. SNP) between individuals that has been linked to a specific trait, such as tenderness, marbling, ribe-eye area (REA), feed efficiency, hot carcass weight (HCW) and yield grade, is known as a DNA marker (Van Eenennam *et al.* 2007; Hall et al. 2011). A DNA marker represents differences in the nucleotide sequence between individuals (Van Eenennam *et al.* 2007). Simple traits, those controlled by a single gene and a marker allele that is associated with that gene, are easily managed to predict an exact phenotype. On the other hand, complex traits, controlled by multiple genes and the environment, are much harder to predict. These traits

may have many gene markers that are associated with multiple genes influencing one specific trait. These traits tend to be the most economically relevant to beef production (Thompson *et al.* 2014).

Specific groups of cattle may be genetically superior for marbling, yield or quality grade while others fatten early or remain lean until late in the finishing period. Managing these cattle based on their growth potential can reduce cost and create product consistency.

2.3. Vitamin A

The intensification of the livestock industry has increased the need to supplement vitamins to meet animal requirements. Along with vitamins D, E and K, vitamin A is an essential fat-soluble vitamin and is one of practical importance in livestock nutrition (NRC 1996). Vitamin A has an important role in reproduction, vision and immunity, as well as adipogenesis (fat deposition).

2.3.1. Structure and Available Forms

The term vitamin A is used to collectively refer to the group of retinoids that exhibit the same biological functions as retinol (ROL). However, ROL is the only retinoid that is considered to be true vitamin A. These retinoids, which include vitamin A precursors and metabolites, belong to a family of polyisoprenoid lipids (Figure 2.2). Structurally, they include a number of isoprene units joined in a chain to a β-ionine ring. Alternating double bonds appear within the side chain. A number of isoforms of vitamin A are formed depending on the chemical group bound to the terminal end of the side chain. For example, ROL contains an alcohol, RAL an aldehyde and RA a carboxyl group (Fig 2.2).

Vitamin A is primarily found in the form of pro-vitamin carotenoids in plant tissue and retinyl esters (RE) in animal tissue (Blomhoff *et al*. 1990; Harrison 2005; Yeum and Russel 2002). The major pro-vitamin carotenoid obtained from plant tissues is β-carotenoid (Eroglu *et al*. 2012), which is also the most common pro-vitamin A

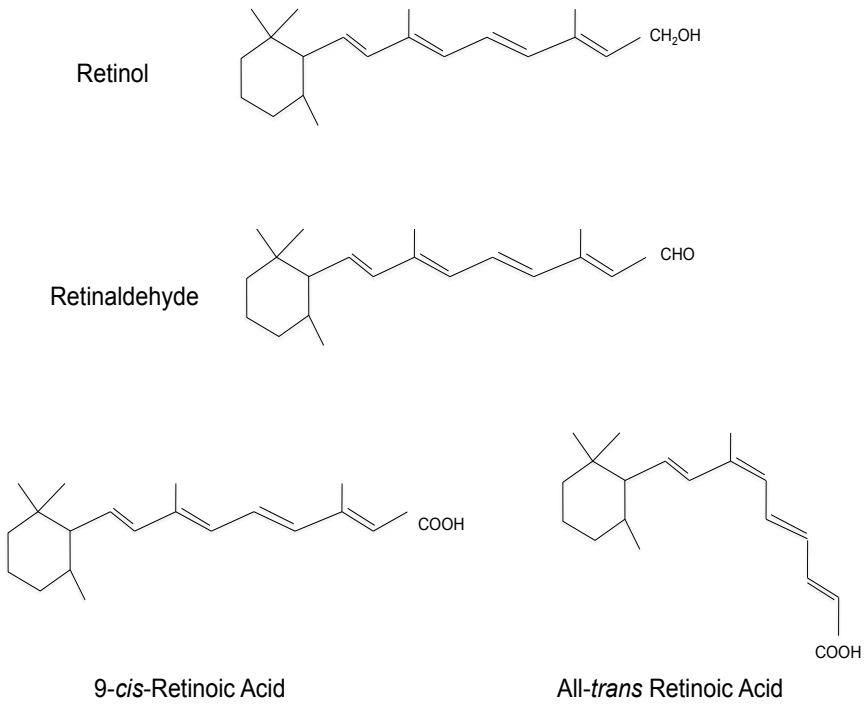

Figure 2.2. Schematic showing the chemical structure of retinol, retinaldehyde and retinoic acid.

found in the ruminant diet and is easily converted to vitamin A within the body (Deming and Erdman 1999). Carotenoids are pigmented molecules belonging to a class of organic compounds termed terpenoids. The skeleton of carotenoids is a 40-carbon (C_{40}) chain, from which the variations of carotenoids arise (Britton 1995).

2.3.2. Absorption and Transportation

Ingested retinyl esters are hydrolyzed to retinol within the intestinal lumen, yielding retinol and a fatty acid, prior to absorption by the enterocytes (Blomhoff et al. 1990; Harrison 2005). In contrast, ingested carotenoids can either remain intact or be cleaved to produce retinol (Harrison 2005). Once the retinol or carotenoid is absorbed into the enterocyte, the retinol is re-esterified to retinyl esters by lecithin:retinol acyltransferase (LRAT) via

reactions with long-chain fatty acids (Blomhoff et al. 1990; Harrison 2005). Due to carotenoids and retinyl esters being hydrophobic molecules, both are incorporated into chylomicrons for transport through the circulatory system (Blomhoff *et al.* 1990; Harrison 2005).

The daily consumption as well as the duration of intake determines the vitamin A status of an individual. As well, the vitamin A status of the individual determines absorption, thus vitamin A absorption does not occur in a linear fashion. Serum concentrations of retinol were maintained at adequate levels for beef steers independent of the level of dietary vitamin A intake (Ward *et al*. 2012; Pickworth *et al*. 2012). Such regulation allows for regular bodily functions to occur (Alosilla *et al*. 2007). However, increasing the number of days with little to no vitamin A supplementation results in a decrease in serum retinol levels (Gorocica-Buenfil *et al*. 2008). Vitamin A readily accumulates in the liver, which is the major storage organ, as well as other tissues, and thus, liver retinol concentrations increase with increasing intake of vitamin A (Bryant *et al*. 2010; Ward *et al*. 2012; Pickworth *et al*. 2012).

Absorbed retinol in the liver can be stored in stellate cells or transported through the circulatory system (Quadro *et al*. 2004). Mobilization of retinol from hepatic tissue requires the secretion of Retinol-Binding-Protein-4 (RBP4), which is a long polypeptide protein chain. Production of RBP4 by hepatic cells is essential for mobilization of retinol from the liver (Quadro *et al*. 2004; Blomhoff 1990), as extra-hepatic production of RBP4 does not play a role in liver retinol transport (Quadro *et al*. 2004).

2.3.3. Metabolism

Within the cytosol, ROL is converted to RAL by the enzyme alcohol dehydrogenase (ADH; Duester 2000) and then it is further oxidized to RA by retinaldehyde dehydrogenase (RALDH; Duester 2000). Alcohol dehydrogenase can catalyze the reverse reaction as well, reducing RAL to ROL (Boleda *et al*. 1993).

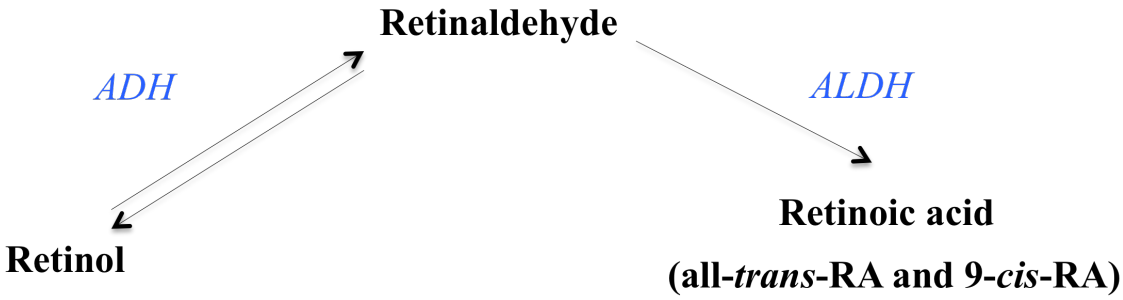

Figure 2.3. Vitamin A (Retinol) metabolism. **ADH**: alcohol dehydrogenase; **ALDH**: aldehyde dehydrogenase.

2.3.4. Role in Adipogenesis

Involving an array of enzymes and adipocyte precursors, adipogenesis is the term used to describe the conversion of preadipocytes into mature adipocytes. It is a complex process. The size of adipose tissue depots increases as the animal ages. In the mature animal the adipose tissue contains multiple cell types with adipocytes being predominant. Adipocyte cells function as storage vesicles for triacylglycerol (Cornelius *et al*. 1994) that can be mobilized during periods of energy deficiency.

Stem cells are the source of adipocytes. Pre-adipocytes are formed through the transdifferentiation of non-differentiated stem cells (Fernyhough *et al*. 2008). Mature adipocytes have the ability to generate new progeny cells that are similar to stem cells, and are able to differentiate (Sugihara *et al*. 1989; Fernyhough *et al*. 2008). In the later stages of adipocyte cell differentiation, RA stimulates fat deposition (Heyman *et al*. 1992), while RAL can cause inhibition (Ziouzenkova *et al*. 2007). This influence is accomplished through interaction with nuclear receptor proteins and the subsequent impact on gene expression (Figure 2.3). Retinoic acid receptor (RAR) and retinoid X receptor (RXR) are nuclear receptor proteins that can interact with RA to form hetero- or homodimers

(Heyman *et al*. 1992; Repa *et al*. 1993; Petkovich 2001; Desvergne 2007). These complexes can then interact with retinoic acid response element (RARE), located in the promoter region of a gene (Heyman *et al*. 1992; Zhang *et al*. 1992). When RA isomers interact with RAR (all-*trans*-RA and 9-*cis*-RA) and RXR (9-*cis*-RA; Heyman *et al*. 1992; Repa *et al*. 1993), the complex can affect gene expression through up-regulation of transcription (Heyman *et al*. 1992; Zouizenkova *et al*. 2007). Retinaldehyde limits fat deposition possibly by acting as an inhibitor of RA activity, through competing as a

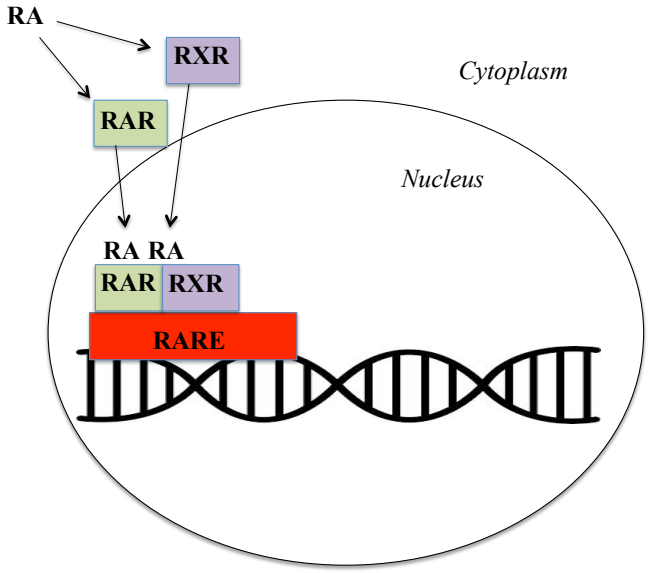

Figure 2.4. Nuclear action of retinoid acid. Retinoic acid (RA) interacts with the nuclear receptors retinoid X receptor (RXR) and retinoic acid receptor (RAR), which then bind to retinoic acid response element (RARE) located within the promoter region of a gene. Subsequently, gene expression is up- or down-regulated.

substrate for RXR and RAR. Retinoid X receptor also has the ability to form heterodimers with other nuclear receptors including peroxisome proliferator-activated receptor (PPARγ; Desvergne 2007; Ziouzenkova *et al*. 2007), which can also interact with vitamin A metabolites to regulate adipogenesis (Ziouzenkova *et al*. 2007).

2.3.5. Effects of Vitamin A Restriction on Production Parameters and Carcass Merit

As discussed previously, vitamin A has an inhibitory effect on fat deposition through the metabolite RAL (Zouizenkova *et al*. 2007). Therefore, restriction of vitamin A in the diet of beef cattle could be an economical way of increasing intramuscular fat (IMF) content by removing the inhibitory effects of vitamin A.

2.3.5.1. Animal Performance

Nutritional studies have examined the effects of vitamin A supplementation level on feedlot cattle performance (Gorocica-Buenfil *et al*. 2007a; Gorocica-Buenfil *et al*. 2008; Gibb *et al*. 2011; Pickworth *et al*. 2012;). Gibb *et al*. (2011) examined the effects of the removal of supplemental vitamin A from beef heifer diets fed barley based rations on animal performance and carcass quality. Animals were allocated to one of two treatments: 0 or 3640 IU/kg DM of vitamin A supplemented within the diet. A decrease in DMI was observed for those animals fed the low vitamin A relative to the high vitamin A treatment. However, other research (Gorocia-Buenfil *et al*. 2007a; Gorocia-Buenfil *et al*. 2007b; Pickworth *et al*. 2012) did not report a reduction in DMI. Despite the reduction in DMI reported by Gibb *et al*. (2011) no difference in ADG was found between the low and high vitamin A treatments. The researchers also reported no significant difference in G:F ratio between the two treatment groups.

2.3.5.2. Intramuscular Fat

Research has shown that vitamin A restriction in the diet of feedlot cattle has a positive impact on IMF deposition (Oka *et al*. 1998; Wang *et al*. 2007; Ward *et al.* 2012), while having no negative impact on cattle performance (Gibb *et al*. 2011; Pickworth *et al*. 2012). Gorocica-Buenfil *et al*. (2007a) examined the effects of vitamin A restriction on marbling content and fatty acid composition in beef cattle when fed diets containing high-moisture (HMC) or dry corn (DC). The Angus-cross steers received either a low vitamin A (no supplemental Vitamin A) or high vitamin A (2,700 IU vitamin A/kg DM) treatment along with either HMC or DC. The animals were kept on feed for 145 days. Results showed that when the steers were fed the low vitamin A treatments,

marbling score and USDA quality grade were higher than those steers fed the high vitamin A treatments. A similar study conducted by Pickworth *et al*. (2012), examined the impact of vitamin A and D restriction on animal performance and fat deposition in feedlot cattle. Cattle fed a diet restricted in vitamin A had higher IMF than those supplemented with vitamin A. No effect of vitamin D restriction on carcass quality was noted.

Ward *et al.* (2012) examined the impact of an interaction between a novel SNP in the gene coding for the enzyme ADH1C and vitamin A supplementation level on IMF deposition in finished beef steers. *Alcohol dehydrogenase 1 C* (ADH1C) is responsible for the conversion of ROL to RAL (Duester 2000), which would increase the availability of RAL for conversion to RA. Retinoic acid has a stimulatory effect on adipogenesis (Ziouzenkova *et al.* 2007). Under vitamin A deficient conditions, the activity of *RALDH1* is increased three-fold, enhancing the biosynthesis of RA from RAL (Napoli *et al.* 1996). Ward *et al.* (2012) then conducted a nutrigenetic investigation into the impact of *ADH1C* genotype variants and vitamin A restriction on IMF deposition. They reported unsupplemented TT steers having 24.4% higher IMF than vitamin A supplemented TT steers, and 22.9% greater IMF than unsupplemented CC steers. The researchers attributed these results to TT steers having higher production of ADH1C, leading to increased RAL available for conversion to RA under vitamin A restricted conditions. Subsequently, this led to increased IMF deposition for these steers. CT and CC steers lacked sufficient ADH1C to enhance conversion of ROL to RAL, and further to RA. Therefore, CT and CC steers experienced limited fat deposition relative to TT animals. The positive impact of the interaction between *ADH1C* genotype and vitamin A supplementation level suggests this SNP could be applied in MAM programs.

2.4.Cattle Marketing

Cattle in Canada are marketed either on a live weight or dressed weight basis. In both cases, the quality grade that is given to each carcass is important in determining the profit for producers. In some markets, particularly in the United States and in grid marketing programs in Canada, a premium is received for producing carcasses with consistently high marbling grades.

2.4.1. Live Weight

Cattle are sold on an average live weight basis, meaning that the price is not set on an individual level, but rather at the pen level. Therefore, the price reflects projected average carcass weight and quality (Parish *et al*. 2009). The packer and the feedlot negotiate a price prior to the finishing period. The cattle are priced on a per hundredweight (CWT) basis.

Example:

Steer weight: 1350 lb

Live price: $120.00/cwt

1350 lb x $120.00 / 100 lb = $1620.00

There is a high level of risk associated with live weight pricing, as the projected carcass quality is not always seen at harvest. There is no differentiation of cattle based on quality, or incentive for improvements through management or genetics as all cattle receive the same price per hundredweight (Parish *et al.* 2009).

2.4.2. Rail Grade

This marketing strategy is based on carcass weight and grade. The producer sells directly to the packer, who sets a standard range for expected dressed weight and grade at the time of harvest. Therefore, the producer is paid based on dressed weight and grade. Discounts are received for carcasses that fall out of the range designated by the packer.

Dressed weight price is calculated based on dressing percentage. If the steer in the previous example dressed out at 60% of live weight, the dressed weight is 972 lb. Therefore, dressed weight price is: $1620 / 972 lb = $1.67/lb carcass weight. Calculation of the final price for the producer takes into account any discounts if carcasses strayed from the optimal range set by the packer.

2.4.3. Grid System

Grid pricing is similar to rail grade, but with emphasis on specific traits. The price of cattle is determined through evaluation of specific carcass merit attributes: carcass weight, yield grade and quality grade (Parish *et al*. 2009; Radunz 2012).

Grid pricing starts with a base price, which is pre-determined by the packer and based on a standard carcass (i.e. Canada AA and Yield Grade 2; DiCostanzo and Dahlen 2000; Parish *et al.* 2009). The base price also specifies discounts for carcasses that do not meet standards, as well as premiums for carcasses meeting or exceeding the set standards (DiCostanzo and Dahlen 2000). The final grid price is calculated after premiums and discounts are added to the base price (DiCostanzo and Dahlen 2000; Parish *et al.* 2009):

Grid Price = base price[1] ± quality grade[2] ± yield grade[3] ± carcass weight[4]

[1]Standard carcass. Specifies optimal range for specific grid.
[2]Premiums or discounts relative to standard carcass quality grade.
[3]Premiums and discounts relative to standard carcass yield grade.
[4]Premiums or discounts relative to standard carcass weight.

2.4.4. Beef Quality Grades

The yield and quality grades of beef carcasses have historically been assigned based on human judgment in the processing facility. Beef carcasses marketed in Canada are assigned a quality grade by the Canadian Beef Grading Agency (CBGA) in accordance with national grading standards (Canada Beef 2012). A carcass undergoes a food safety inspection, and subsequently subjected to a rigorous assessment process for quality before a quality grade is assigned. Factors that are considered in this process are sex, maturity, meat color, fat color, muscling, fat cover and texture, meat texture and marbling level (Alberta Beef 2001; Canada Beef 2012).

Marbling is one of the major factors determining quality grade (Figure 2.4). It is defined as the intramuscular fat, or the fat found between muscle fibers in skeletal muscle. This is different from intermuscular fat

or seam fat, which is located between muscles. Marbling is associated with increased perceived tenderness as well as juicieness and flavour (Johnston 2001).

The marbling potential of an individual animal is influenced by multiple factors, including: genetics (Melucci *et al*. 2012), nutritional factors such as the energy content of the ration (Faulkner *et al*. 1994; Wertz *et al*. 2002), management factors including hormonal implants, days on feed, backgrounding; (Bruns *et al*. 2005; Preston 1999) as well as the surrounding environmental (Kreikemeier *et al*. 1998). All of these parameters should be taken into consideration for the development of an integrated management approach for finishing beef cattle.

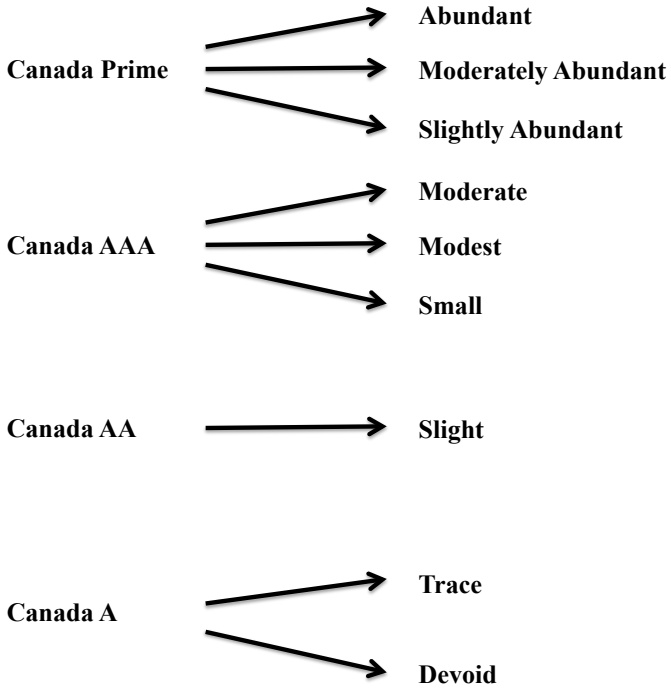

Figure 2.5. Diagram outlining the allowable level of intramuscular fat within each Canadian quality grade for beef cattle.

2.5. Conclusion

Consumer demand is the driving force governing the beef industry today, with the consumer placing a significant amount of emphasis on meat quality. As a result, consumers and restaurants alike have increased the demand for carcasses with higher marbling scores. Subsequently, beef producers have adjusted to these changing demands in product quality, and shifted production towards value based and niche markets. Marker assisted management programs are one way that beef production has applied to fill these niche markets.

The identification of DNA markers associated with economically relevant traits such as marbling or tenderness, allows the segregation of genetically superior animals, which results in lower production costs and maximized value per animal. These DNA markers enable strategic implant programs, ration formulation and disease prevention along with the development of brands such as Certified Angus Beef® or Sterling Silver®. The development of brands such as these has allowed producers to build populations of cattle based on genetics, and manage these cattle groups to meet consumer demand for specific traits.

The effect of *ADH1C* genotype and vitamin A supplementation level on intramuscular fat deposition has the potential for use in a MAM program. However, an optimal supplementation level must be determined prior to application of this technology in the beef industry.

3. HYPOTHESIS

Previous research indicates an improvement in intramuscular fat for beef cattle when fed restricted levels of vitamin A during finishing. The discovery of a novel SNP in the *ADH1C* gene (*ADH1C c.-64T>C*) suggests an interaction between nutrition and genetics whereby, steers TT at the SNP deposit increased levels of IMF under vitamin A restriction relative to CC animals.

1) In the present study, we hypothesize that intramuscular fat will be improved when vitamin A is supplemented between the levels of 0 and 75% of the NRC recommended 2200 IU/kg DM.

2) When vitamin A is supplemented within this range, genotypic differences at *ADH1C c.-64T>C* will result in phenotypic variation in IMF whereby TT animals will deposit greater IMF relative to CC animals.

4. EFFECT OF VITAMIN A SUPPLEMENTATION LEVEL AND *ADH1C* GENOTYPE ON MARBLING IN BEEF STEERS

4.1. Introduction

The beef cattle industry today is driven largely by consumer demand and preference. Marbling (intramuscular fat (IMF)) is a major factor linked to consumer satisfaction through increased juiciness and flavor as well as perceived tenderness of the meat (Johnston 2001). In grid marketing programs in Canada and the United States, a premium is received for producing carcasses with consistently high marbling grades (DiCostanzo and Dahlen 2000). An emerging method of improving carcass consistency is through Marker-Assisted Management (MAM), which involves management of cattle to improve production and carcass traits based on the genotype of the animal.

Previous nutritional studies have shown that vitamin A restriction in the diet of feedlot cattle has a positive impact on marbling (Oka *et al.* 1998; Wang *et al.* 2007), while having no negative effect on average daily gain (ADG) or feed to gain ratio (F:G; Gibb *et al.* 2011; Pickworth *et al.* 2012). A high serum retinol concentration is negatively associated with marbling (Oka *et al.* 1998).

Increased IMF as a result of vitamin A restriction can be partially explained through nutrigenomics, whereby a bioactive component of feed regulates gene expression (Marti *et al.* 2010). Retinol (ROL; vitamin A) is oxidized to retinaldehyde (RAL) within the cytosol by alcohol dehydrogenase (ADH; Duester 2000), and then further oxidized to retinoic acid (RA) by aldehyde dehydrogenase (ALDH; Duester 2000). Both RAL and RA have the ability to regulate a number of genes involved in adipogenesis (Ziouzenkova *et al*. 2007). Specifically in the later stages of adipocyte cell differentiation, RA stimulates fat deposition (Heyman *et al.* 1992), while RAL has an inhibitory effect (Ziouzenkova *et al*. 2007).

A recent study by Ward *et al.* (2012) reported a novel SNP in the promoter region of the *alcohol dehydrogenase 1C* (*ADH1C c.-64T>C*) gene, which codes for the enzyme ADH1C. They reported that animals TT at the SNP had higher IMF than steers CC at the SNP when vitamin A was removed from the ration. No association was observed when steers were fed NRC recommended vitamin A levels. The steers used in that study were also fed a vitamin A deficient backgrounding ration to reduce liver retinol stores prior to the finishing period. However, complete restriction of vitamin A is impractical from a commercial standpoint due to potential production and health ramifications of clinical vitamin A deficiency (NRC 1996).

The interaction between *ADH1C c.-64T>C* and vitamin A supplementation level indicates there is potential use of this SNP in MAM programs. The objective of the current research was to determine the optimum vitamin A supplementation level, in combination with *ADH1C* genotype, for beef cattle diets in order to improve carcass quality grade.

4.2. Materials and Methods

All animals were cared for according to the guidelines outlined by the *Guide to the Care and Use of Experimental Animals* (Canadian Council on Animal Care 1993).

4.2.1. Animal Selection and Housing

Initially, four hundred and fifty crossbred steers, black in color, were purchased from a commercial auction market. At the time of processing, a hair sample from the tail of each animal was obtained and used for DNA extraction and genotyping. The steers were genotyped for *ADH1C c.-64T>C* using a PCR-RFLP modified from Ward *et al.* (2012). Briefly, 25 µL PCR cocktail (0.2 pmol forward and reverse primers (Ward *et al.* 2012; Integrated DNA Technologies, Coralville, IA), 0.2 mM dNTP (Fermentas, Burlington, ON), 0.5 mg/ml BSA (New England Biolabs, Pickering, ON), Taq polymerase (Thermo Fisher Scientific, Waltham, MA), 10X Taq buffer with $(NH_4)_2SO_4$ (Thermo Fisher Scientific, Waltham, MA), 2 mM $MgCl_2$ (Thermo Fisher Scientific, Waltham, MA)

was mixed with 1.5 µl DNA. The PCR product, which was 250-bp in length, was digested for five hours with *BslI* (New England Biolabs, Pickering, ON). The resulting fragments were separated using electrophoresis with a 3% agarose gel stained with ethidium bromide. Forty-five TT, 45 CT and 27 CC animals were selected for a total of 117 steers and included in the feeding trial. The steers were housed in four pens with an even distribution of genotype and treatment across pens. In the current trial, no hormonal implants were used in order to eliminate any negative effects on marbling potential (López-Campos *et al.* 2013; Duckett *et al.* 1999).

4.2.2. Diets and Treatments

The trial was conducted at the University of Saskatchewan's Beef Cattle Research Facility. The 117 steers (327 ± 52 kg) were backgrounded for 66 days on a commercial backgrounding ration (Table 4.1). This phase was designed to mimic commercial industry

Table 4.1. Composition and nutrient analysis of backgrounding and finishing rations on a dry-matter basis.

	Backgrounding	Finishing
Diet Composition, % DM		
Barley Silage	9.71	2.22
Grass Hay	15.70	-
Barley Grain	44.96	82.36
Canola Meal	-	5.26
Wheat DDGS	9.2	-
Barley Straw	14.05	4.83
Supplement[1]	6.39	5.32
Nutrient Analysis, % DM		
Total digestible nutrients	65.4	75.1
Crude protein	12.3	13.4
Acid detergent fiber	23.1	10.1
Neutral detergent fiber	37.6	22.1
Ca	0.7	0.6
P	0.4	0.4
Vitamin A (IU/kg DM)[2]	3664	555

[1]Supplement supplied by Federated Co-operative Ltd., Saskatoon. SK. Contained: 540 ppm Zn, 499 ppm Mn, 182 ppm Cu, 16 ppm I, 4.60 ppm Co, 1.34 ppm Se, 5000 IU/kg Vitamin D3, 500 IU/kg Vitamin E, 620 mg/kg Rumensin® Premix 200 (Elanco, Guelph, ON).
[2]Calculated as 400 IU/mg β-carotene (NRC 1970).

practices. The basal diet provided 3664 IU vitamin A/kg DM, which is above the 2200 IU/kg DM recommended by NRC (1996). Therefore, no vitamin A was supplemented during the backgrounding period. Representative samples of barley grain and hay were sampled and analyzed for β-carotene content (DSM Nutritional Products Inc., Ayr, ON) prior to inclusion in the ration. Barley silage samples were collected every 21 days throughout the trial period for analysis of β-carotene (DSM Nutritional Products Inc., Ayr, ON).

Following completion of the backgrounding period, the animals were adapted to an 83% barley grain finishing ration (DM basis) that contained no supplemental vitamin A (Table 4.1) and fed over a 5 month period. The basal ration contained 555 IU/kg DM of vitamin A. Three vitamin A treatments were provided, supplemented to meet 25, 50 and 75% of the animal's daily requirements as outlined by NRC (1996). Vitamin A supplementation was based upon vitamin A level in the basal diet, animal weight and expected dry matter intake (DMI). Gelatin capsules (Torpac, Fairfield, NJ) were used to administer the vitamin A treatments via oral bolusing every 28 days. The capsules were filled with sugar (0% supplementation) or sugar plus microencapsulate retinyl palmitate (1,000,000 IU/g, Adisseo, Antony, France).

4.2.3. Chemical Analysis

Total mixed ration (TMR) samples were collected once a week and composited bi-weekly. The composite samples were ground through a 1 mm screen using a hammer mill (Christie-Norris Laboratory Mill, Christie-Norris Ltd. Chelmsford, UK) and sent to Cumberland Valley Analytical Services (CVAS, Hagerstown, MD) for analysis according to the methods outlined by the Associate of Official Analytical Chemists (2000). Briefly, the samples were analyzed for: DM (AOAC 2000; method 930.15), crude protein using a Leco Fp-528 Nitrogen Combustion Analyzer (Leco, St Joseph; AOAC 2000; method 984.13) and ADF (AOAC method 973.18). Neutral detergent fiber was analyzed using the method outlined by Van Soest et al. (1991).

4.2.4. Animal Performance Data

The animals were provided feed *ad libitum* once daily and the amount of feed provided per pen was recorded daily. Feed bunks were cleaned once every two weeks and the orts weighed. The amount of feed fed (DM basis), adjusted for orts (weigh back), was used to calculate the average daily dry matter intake for each pen.

Weights of the animals were recorded every two weeks for the duration of the finishing period. At the start and end of finishing, ultrasound measurements of the *longissimus thoracis* (LT) muscle and backfat were recorded using the techniques outlined by Bergen *et al.* (1996).

4.2.5. Carcass Characteristics

Following completion of the finishing period, the animals were slaughtered at a commercial abattoir in three groups over a two week period. Hot carcass weight (HCW) was obtained along with a 19 mm steak collected from the LT muscle between the 12^{th} and 13^{th} rib from each carcass. Marbling was measured by a Canadian Beef Grading Agency (CBGA) trained grader. Following grading, the subcutaneous fat was trimmed off of each steak and the samples vacuum packaged and stored at -20 °C until analysis.

Quantification of intramuscular fat was measured via a petroleum ether extraction method (AOAC 1990, method 960.39). Each steak was ground to produce a homogenous mixture. Duplicate samples of 3 g were weighed, mixed with sand (Alfa Aesar, Ward Hill, MA) to prevent clumping and placed in a cellulose extraction thimble (Whatman International Ltd., Maidstone, England). The thimble and sample were then placed in an extraction beaker and dried overnight in a forced air oven at 105°C. The following morning the samples were extracted for six hours with petroleum ether (Fischer Scientific Ottawa, ON). Following extraction the ether was allowed to evaporated overnight, after which the samples were dried in a forced air oven for one hour at 105°C and weighed. Intramuscular fat was reported as a percentage of extracted fat of the LT using the following equation:

$$\% \text{ Fat} = \frac{(\text{Beaker + Fat weight}) - (\text{Beaker weight})}{\text{Weight of Sample (DM basis)}} \times 100$$

4.2.6. Intensive Sub-population Sampling

A subpopulation of 45 animals (15 TT, 15 CT and 15 CC) was randomly selected from the test population for more intensive sampling during the finishing phase. At the start and end of the trial, a liver biopsy sample as well as a blood sample was taken from each of the 45 intensive steers for analysis of vitamin A status. The blood sample was collected via jugular venipuncture in 10 ml untreated Vacutainer tubes (BD Diagnostics, Plymouth, UK). The samples were immediately protected from light and sent to Prairie Diagnostic Services (Saskatoon, SK, Canada) for analysis of serum retinol concentration. A custom-made biopsy instrument, with an internal diameter of 5 mm, was used to collect the liver samples. The steers were sedated with 0.1 mg/kg body weight (BW) xylazine (Bayer, Toronto, ON), and given pre-emptive analgesia via an intramuscular injection of 1.5 ml/50 kg BW Anafen (ketoprofen; Merial, Baie D'Urfe, QC). The biopsy site, located between the 12^{th} and 13^{th} ribs, was shaved and disinfected three times with Dovidine (Laboratoire Atlas Inc., Montreal, Quebec) and 70% isopropanol (Rougier Pharma, Mirabel, QC). Subcutaneous injection of 6 ml 2% lidocaine with epinephrine (Bimeda MTC, Cambridge, ON) was then given as a local anaesthesic. A small incision was made between the 12^{th} and 13^{th} rib with a #15 blade scalpel and the biopsy instrument inserted into the abdominal cavity for collection of the liver sample. Approximately 1 g of liver was preserved on ice and protected from light prior to being taken to Prairie Diagnostic Services (Saskatoon, SK, Canada) for analysis of vitamin A content. The remainder of the liver biopsy was placed on ice and then frozen at -80°C until analysis for ADH1C protein quantification.

4.2.7. Protein Quantification

A Total Protein Extraction Buffer kit (VWR International, Edmonton, AB) was used for protein extraction from the liver samples, with total protein concentration in each sample determined using a MicroLowry Kit (Sigma-Aldrich, Oakville, ON).

Western Blot analysis was performed to evaluate ADH1C protein abundance in hepatic tissue. β-actin expression was used as a loading control. 10 percent acrylamide resolving gels (1.5 ml 4x lower gel buffer (1.5 M Tris, 0.4% w/v SDS), 2.0 ml of 30% acrylamide/bis-acrylamide mix (29.2% w/v acrylamide and 0.8% w/v bisacrylamide), 2.5 ml ddH2O, 96 µl 10% ammonium persulfate and 6 µl TEMED) were used with stacking gels at 1 mm for ADH and 1.5 mm for β-actin. The wells were stained with 1x SDS buffer. SDS-PAGE was performed for one hour at a constant 180V. Pageruler Plus (prestained protein ladder; Thermo Fisher Scientific, Waltham, WA) was used as the gel ladder. Protein was transferred to a Whattman Protran BA 85 nitrocellulose membrane (GE Healthcare) at 25V for 20 minutes using a Trans-Blot Turbo transfer system (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked for one hour with PBS + 5% skim milk powder. Following this, the membranes were incubated overnight at 4°C with the primary antibody (mouse ADH antibody (sc-137078), Santa Cruz Biotechnology, Inc., Dallas, TX) and mouse β-actin antibody ((sc-47778), Santa Cruz Biotechnology, Inc., Dallas, TX) at a concentration of 0.5 ug/ml in PBS + 5% milk + 0.1% Tween. Membranes were washed three times in PBS + 0.1% Tween, after which the infrared 680 nm dye-tagged secondary antibody (Odyssey Goat anti-Mouse IR Dye 680, Mandel Scientific) was added at a concentration of 1:5000. The membranes were allowed to nutate at 20°C for one hour and washed three times. Membranes were visualized using a LICOR Odyssey infrared imager and protein expression was quantified using Odyssey version 2.0 software.

4.2.8. Statistical Analysis

Data was analyzed using the mixed model procedure of SAS 9.3 (SAS Version 9.3; SAS Institute, Inc., Cary, NC) as a 3 x 3 (3 genotypes x 3 treatment) factorial design. The individual animal was used as the experimental unit. A Kenward Roger adjustment was used to adjust standard errors and means were separated using Tukey's LSD. Significance was set at $P<0.05$ and trends at $P<0.10$.

4.3. Results and Discussion

4.3.1. Serum and Liver Retinol Concentrations

Serum retinol was affected by time of sampling and vitamin A treatment level (Table 4.2). Over the course of the trial, steers supplemented at 75% of NRC (1996) guidelines had higher ($P<0.0001$) serum retinol compared to steers supplemented at 25% and 50% of NRC (1996; Figure 4.1). Similar results have been shown in several studies, where steers receiving no supplemental vitamin A had decreased serum retinol relative to steers fed at the NRC recommendations or higher for vitamin A (Ward *et al.* 2012; Pickworth *et al.* 2012). Serum retinol was significantly greater ($P<0.0001$) at the start of the trial than at the end of the trial. These results are consistent with previous research on vitamin A restriction in beef cattle diets (Gorocica-Buenfil *et al.* 2008; Gorocica-Buenfil *et al.* 2007a). Although the levels decreased over the trial period (Figure 4.2), they were maintained at levels considered normal for feedlot cattle (>20 μg/dL; Puls 1994). No evidence of clinical vitamin A deficiency was detected throughout the trial.

Table 4.2. Effect of vitamin A treatment, *ADH1C c.-64T<C* genotype and time on serum and liver retinol concentrations during the finishing period. Values in bold indicate significance. Significance declared at P<0.05.

Effect	Serum Retinol	Liver Retinol
Treatment	**<0.001**	0.323
Genotype	0.439	0.886
Time	**<0.001**	**<0.001**
Treatment x Time	0.760	0.384
Treatment x Genotype	0.905	0.465
Genotype x Time	0.932	0.977
Treatment x Genotype x Time	0.938	0.407

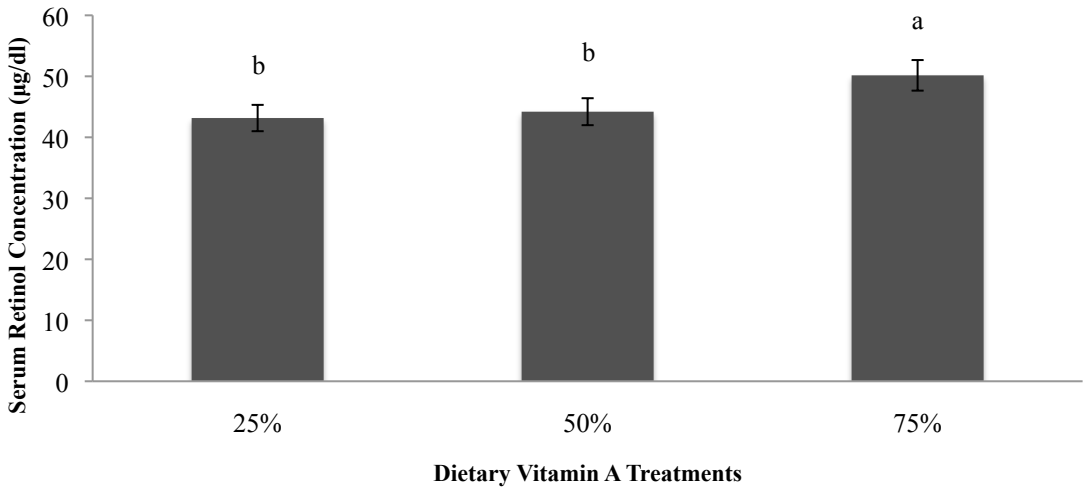

Figure 4.1. Serum retinol concentrations (µg/dl) obtained from steers in the subpopulation on each of the supplemental vitamin A treatments. Bars with differing superscripts are significantly different (P<0.05). Error bars indicate the SEM.

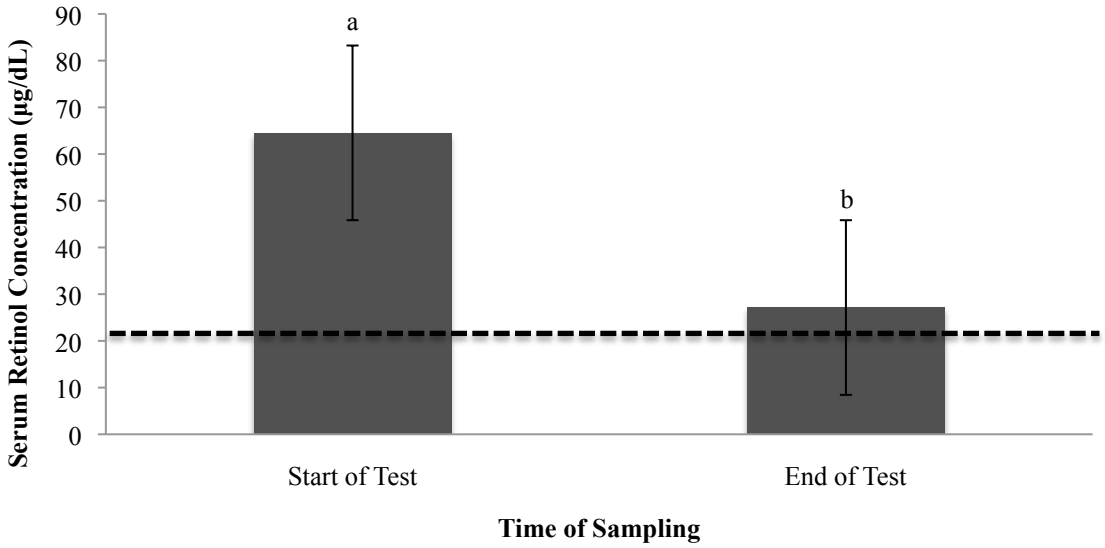

Figure 4.2. Serum retinol (µg/dl) measurements obtained at the start and end of the finishing period the 45 steers selected for more intensive sampling. Bars with differing superscripts are significantly different ($P<0.05$). The dashed line indicates high marginal serum retinol concentrations for cattle, with value below being deficient and values above being adequate (Puls 1994). Error bars indicate the SEM.

Regardless of treatment or genotype, liver retinol concentrations were higher ($P<0.0001$) at the start of the finishing period relative to the end of finishing (Table 4.2; Figure 4.3). Lower liver retinol levels at the end of the trial was expected as all steers were fed restricted levels of vitamin A over the 5-month feeding period and were utilizing stored vitamin A. These values are below the threshold for low marginal liver retinol stores (<30 ppm; Puls 1994), indicating vitamin A deficiency. This is reflected in the lower serum retinol levels observed at the end of the trial as the liver is responsible for attenuating serum retinol status when the dietary level of vitamin A is restricted (Blaner and Olson 1994). Pickworth *et al.* (2012) also reported levels as low as 2 µg/g for beef steers following 184 days on feed with vitamin A restriction. Serum and liver samples in the current trial were collected four weeks following administration of the vitamin A treatments. Therefore, it would be expected that steers would have a lower concentration of retinol in the serum and liver as they have been utilizing the available retinol throughout the four-week period.

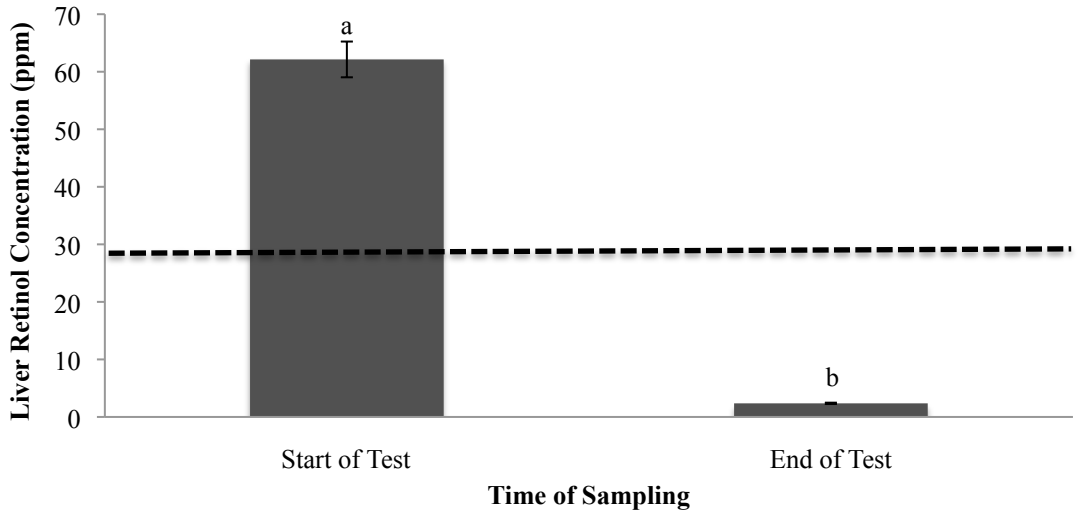

Figure 4.3. Liver retinol (ppm) concentrations obtained at the start and end of finishing from the 45 steer subpolulation. Bars with differing superscripts are significantly different ($P<0.05$). The dashed line indicates low marginal liver retinol levels in cattle (Puls 1994). Error bars indicate the SEM.

During the finishing period, silage samples were collected monthly for β-carotene analysis and vitamin A supplementation in each treatment were adjusted accordingly. The level of β-carotene in the silage overall during the trial period was 43.6 mg/kg (17,520 IU/kg DM). However, the vitamin A level dropped from 48.5 mg/kg (19,400 IU/kg DM) to 32.8 mg/kg (13,120 IU/kg DM) between the start and end of finishing. The vitamin A content in feed decreases with the length of storage, exposure to sunlight or heavily processed feeds (NRC 1996). Pickworth *et al.* (2012) reported a 39% reduction in vitamin A equivalents in corn silage over a 3-month period. Both pre- and post-harvest conditions also impact the carotenoid content in feeds (Pickworth *et al.* 2012; Aurand *et al*. 1947). Ruminal degradation of retinoids also increases with high concentrate rations compared to forage diets (Rode *et al.* 1990), leading to a reduction in absorbed vitamin A. Although liver retinol was deficient by the end of finishing, serum concentrations were sufficient to maintain homeostasis and prevent observation of clinical vitamin A deficiency.

4.3.2. Feedlot Performance

The effects of feeding the three dietary vitamin A levels on performance and carcass parameters of finished beef steers are shown in Table 4.3. There was a trend for increased final finishing weight ($P=0.081$) and ADG ($P=0.096$), as well as a significant increase in HCW ($P<0.001$) for steers fed the 25% vitamin A treatment relative to the other two treatments. Previous research observed no difference in performance parameters such as DMI, ADG or final finishing weight when dietary vitamin A was restricted (Gorocica-Buenfil *et al.* 2008; Pickworth *et al.* 2009; Bryant *et al*. 2010; Ward *et al*. 2012; Pickworth *et al.* 2012).

The first signs of vitamin A deficiency include decreased DMI and ADG, followed by a rough hair coat, slow growth, edema and blindness (NRC 1996). These symptoms were not observed in any of the steers in the current trial, therefore, there were no observable signs of clinical vitamin A deficiency at the conclusion of the trial.

Table 4.3. Effect of vitamin A treatment and *ADH1C c.-64T>C* genotype on production and carcass parameters. Values in bold indicate significance while italics indicates a trend. Significance declared at P<0.05, trends at P<0.1.

	Genotype			Treatment[1]				P-value		
Parameter	TT	CT	CC	25	50	75	SEM	G	T	G*T
BW, kg										
Start of BG[2]	326.6	321.4	317.3	324.5	316.0	324.7	1.671	0.27	0.22	0.53
Start of finish	399.3	392.8	392.7	400.6	388.4	395.8	2.230	0.42	0.15	0.56
End of finish	584.5	588.6	586.9	595.4	577.4	587.2	6.102	0.85	*0.08*	0.89
ADG, (kg/d)										
BG	0.9	0.9	0.9	1.0	0.9	0.9	0.017	0.94	0.40	0.35
Finish	1.4	1.4	1.4	1.5	1.4	1.4	0.014	0.68	*0.09*	0.71
LTA, (cm^2)										
Start of finish[3]	49.6	48.7	48.9	50.4	47.6	49.2	0.532	0.75	0.11	0.74
End of finish[3]	87.5	86.3	86.1	87.2	85.2	87.5	0.620	0.61	0.28	0.17
Grader[4]	79.7	77.9	78.9	81.7a	75.7b	79.1a	0.776	0.61	**<0.001**	0.85
Backfat, mm										
Start of finish[3]	3.3	3.2	2.8	2.9	3.1	3.1	0.146	0.35	0.90	0.41
End of finish[3]	10.4	10.7	10.1	9.9	10.8	10.4	0.205	0.50	0.22	0.99
Marbling score										
Ultrasound[3]	5.5	5.4	5.4	5.5	5.4	5.4	0.052	0.52	0.48	0.54
Grader[4,5]	6.2	5.8	5.9	6.1	5.8	5.9	0.098	0.43	0.48	0.43
HCW, (kg)	331.2	333.5	325.9	337.9a	322.8c	329.6b	1.941	0.31	**<0.001**	0.78
Average fat, (mm)	13	13	13	13	13	13	0.277	0.61	0.68	0.76
Grade fat, (mm)	12	13	12	12	13	13	0.300	0.75	0.46	0.85
Lean yield[6], (%)	55.9	55.3	55.9	56.4	55.2	55.6	0.277	0.44	0.22	0.83
Intramuscular fat, (%)	6.6	6.1	5.9	6.4	6.2	5.9	0.207	0.33	0.74	**0.04**

[1]Supplemented as a percent of 2200 IU/kg DM (NRC 1996).
[2]BG – Backgrounding.
[3]Measured by ultrasonography.
[4]Measured by a Canadian Beef Grading Agency certified grader.
[5]Measured using the USDA ten-point scale where 1.0 is devoid of marbling and 10.0 is abundant.
[6]Calculated according to the CBGA equation: 57.96 – 0.027 HCW, kg, + 0.202 rib-eye area, cm^2, - 0.703 average fat, cm.
[ab]Means within the same row with differing superscripts are significantly different (P<0.05).

4.3.3. Carcass Characteristics

Area of the LT muscle, as determined by a Canadian Beef Grading Agency certified grader was greater ($P<0.001$) for steers receiving the 25% or 75% vitamin A treatment than the 50% treatment (Table 4.3). This result, along with a significantly higher HCW ($P<0.001$), is a consequence of a trend observed for increased ADG for steer on the 25% treatment. Previous research does not report an impact of restricted vitamin A levels in beef cattle diets on feedlot performance, as well as indicating no inhibitory effects of high supplementation on growth parameters (Gibb *et al*. 2011; Pickworth *et al*. 2012). Further research should investigate any potential impact of high levels if vitamin A supplementation on beef cattle growth.

Genotype and vitamin A treatment had no impact on marbling score or ether extracted IMF of the LT muscle. These results are not consistent with previous research (Gorocica-Buenfil *et al.* 2007ab; Bryant *et al*. 2010; Gibb *et al.* 2011; Ward *et al.* 2012; Pickworth *et al.* 2012) who reported an increase in marbling scores in beef steers when vitamin A was restricted. However, a genotype x treatment interaction ($P=0.04$) was observed for ether extractable lipid from the LT muscle (Table 4.3; Figure 4.4). Within the 75% vitamin A supplementation treatment, TT steers had higher IMF relative to CT and CC animals. This pattern was not observed for the two lower vitamin A supplementation levels.

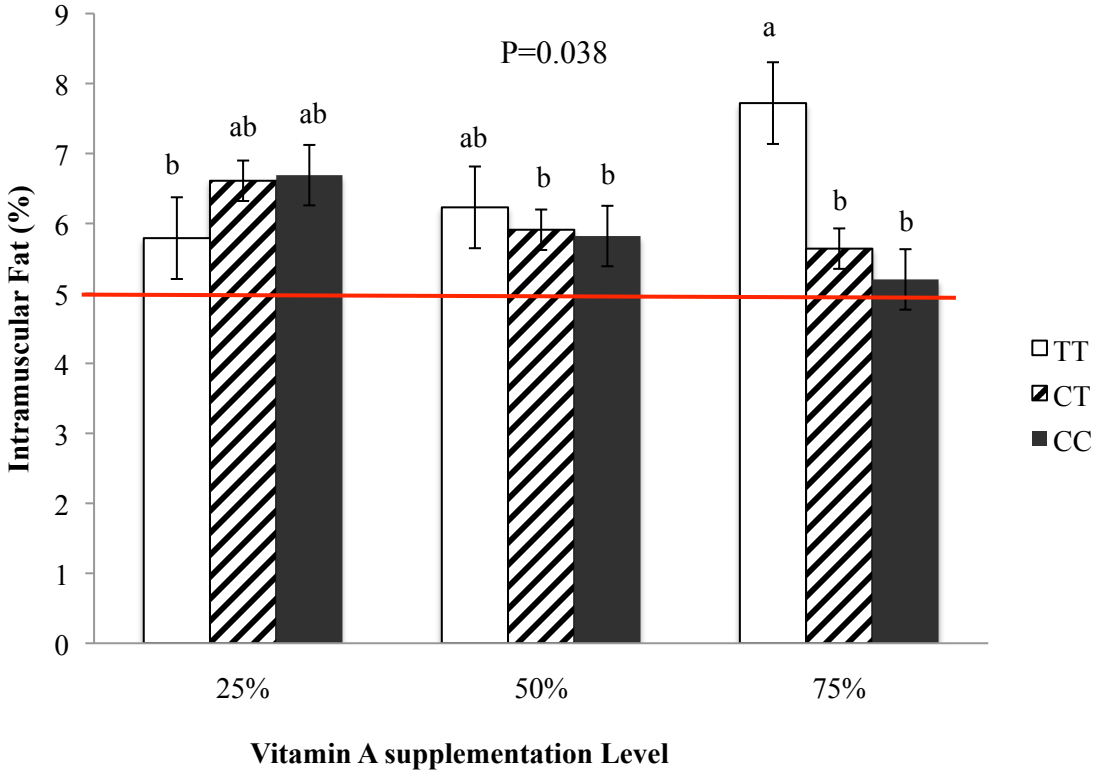

Figure 4.4. Intramuscular fat (IMF) of the *longissimus thoracis* (LT) muscle of TT, CT and CC steers on each of three vitamin A treatments (25%, 50%, and 75% of NRC recommendation of 2200 IU vitamin A/kg DM). The red line indicates the cutoff for carcass grades of AA and AAA, with bars above the line grading AAA. Bars with differing subscripts are significantly different ($P<0.05$). Error bars indicate SEM.

Previously, Ward *et al.* (2012) reported that unsupplemented TT steers had 24.4% higher IMF than supplemented TT steers, and 22.9% greater IMF than CC steers unsupplemented. It is unclear as to why TT animals had increased IMF at the higher supplementation level relative to the lower supplementation treatments in the current trial. However, it should be pointed out that regardless of treatment or genotype, 84% of steers graded Canada AAA (USDA Choice), indicating a lack of variation in IMF and point to the possibility that all animals were finished beyond the point where a genotype effect would have been seen.

The results for the cattle fed 25% and 50% of NRC recommendations, regardless of genotype, agree with previous research, where vitamin A restriction in the diet of finishing beef steers increased IMF deposition (Oka *et*

al. 1998; Wang *et al.* 2007; Gorocica-Buenfil *et al.* 2007ab; Gibb *et al.* 2011; Ward *et al.* 2012). The mechanism responsible for this increase is likely based on the role of vitamin A in adipogenesis. The vitamin A metabolites RAL and RA regulate adipogenesis through interaction with nuclear receptor proteins. The enzyme ADH1C oxidizes ROL to RAL (Duester, 2000). Accumulation of RAL has been found to have an inhibitory effect on adipogenesis (Ziouzenkova *et al*. 2007). During periods of vitamin A restriction, as was seen in the current trial, the reduced consumption of vitamin A decreases the availability of ROL for conversion to RAL. Subsequently, the inhibitory effect of RAL on adipogenesis is removed leading to the increase in IMF observed in the current study. The failure to see a genotype effect at these lower levels of vitamin A supplementation may, as indicated earlier, be due to the fact that all cattle were finished past the point were a phenotypic effect of genotype was able to be seen. Further research should look at the effect of varying days on feed for beef steers on the interaction between *ADH1C* genotype and vitamin A supplementation level on carcass traits.

In contrast, on the 75% vitamin A treatment, IMF was higher for TT steers relative to CT and CC animals (Figure 4.4). Animals TT at the SNP have increased mRNA expression of *ADH1C* relative to CC animals (Ward *et al.* 2012). This should result in an increased production of ADH1C in TT steers, leading to a higher conversion rate of ROL to RAL. Under vitamin A deficient conditions, the activity of *RALDH1* is increased three-fold (Napoli *et al.* 1996). As is evident from Figure 4.3, liver retinol levels dropped significantly by the end of the trial. As a result of this *RALDH1* activity would be expected to increase, thus enhancing the biosynthesis of RA from RAL (Napoli *et al.* (1996). Retinoic acid has a stimulatory effect on adipogenesis (Ziouzenkova *et al.* 2007). Therefore, although TT steers had an increased production of RAL, the further conversion to RA would also be enhanced leading to an increase in IMF deposition. In contrast CT and CC steers had a reduced conversion rate of ROL to RAL due to low levels of *ADH1C*, thus limiting the availability of RAL for conversion to RA. Subsequently, IMF deposition was limited in these animals relative to TT steers.

Ward *et al.* (2012) proposed that the association between *ADH1C* genotype and vitamin A supplementation level would only be observed when vitamin A was limiting. However, these researchers primed the steers with a vitamin A deficient backgrounding diet prior to the finishing period, which was done to reduce liver retinol stores and ensure that variation between treatment and genotype would be observed. In the current trial, steers were fed a commercial backgrounding ration for 66 d containing 1.7 times the NRC recommendation of 2200 IU/kg diet DM (NRC 1996). Future research should investigate the possible impact of the length of vitamin A restriction may have on the effect of *ADH1C* genotype on IMF deposition.

4.3.4. Protein Quantification

Genotype and time had an impact ($P<0.05$) on hepatic ADH1C levels (Figure 4.5; 4.6), while there was no impact of vitamin A treatment. The ADH1C enzyme levels were higher ($P=0.0176$) in TT steers compared to CT and CC animals. The T allele creates a binding site motif for the transcription factor C/EBPα (Chekmenev *et al.* 2005), resulting in increased mRNA abundance in TT steers (Ward *et al*. 2012). Previously, Ward *et al.* (2012) reported an additive effect of *ADH1C* mRNA expression in hepatic tissue with TT steers displaying significantly greater expression than CC steers. Animals with both the C and T allele were intermediate. There researchers suggested that the increased expression of *ADH1C* would translate into higher ADH1C enzyme production. The results of the current trial affirm this pathway.

Retinol is converted to RAL by the enzyme ADH1C (Duester 2000). Increased hepatic ADH1C in the current trial suggests an increased conversion of ROL to RAL for TT animals. As indicated earlier, under vitamin A restricted conditions, the activity of RALDH1 is increased (Napoli *et al.* 1996), resulting in the further oxidation of RAL to RA. Retinaldehyde can inhibit adipogenesis, while RA has a positive impact (Ziouzenkova *et al.* 2007). Therefore, the higher ADH1C levels in hepatic tissue for TT steers should result in a higher conversion rate of ROL to RAL, and further to RA, resulting in increased stimulation of adipogenesis (Ward *et al.* 2012). This was seen in steers on the 75% vitamin A treatment level. However, as discussed previously, since over 80% of the

steers received quality grades of AAA or higher, there was a lack of variation within treatments to see the effects of varying protein production levels between the genotype variants, particularly at the 25% and 50% vitamin A treatments.

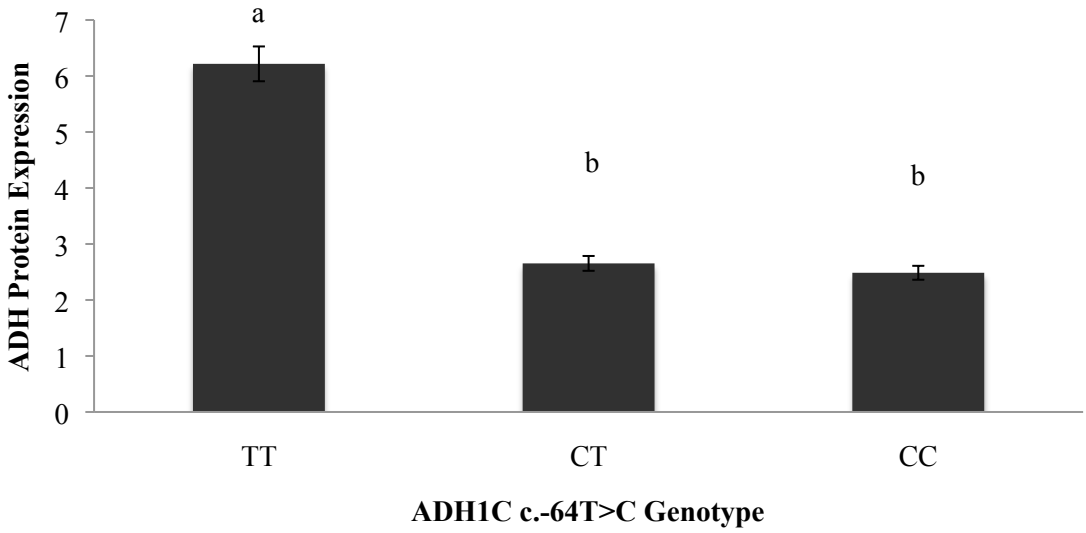

Figure 4.5. Relative expression of ADH1C from hepatic tissue obtained from steers with differing *ADH1C c.-64T>C* genotypes. Bars with differing subscripts are significantly different (P<0.05). Error bars indicate the SEM.

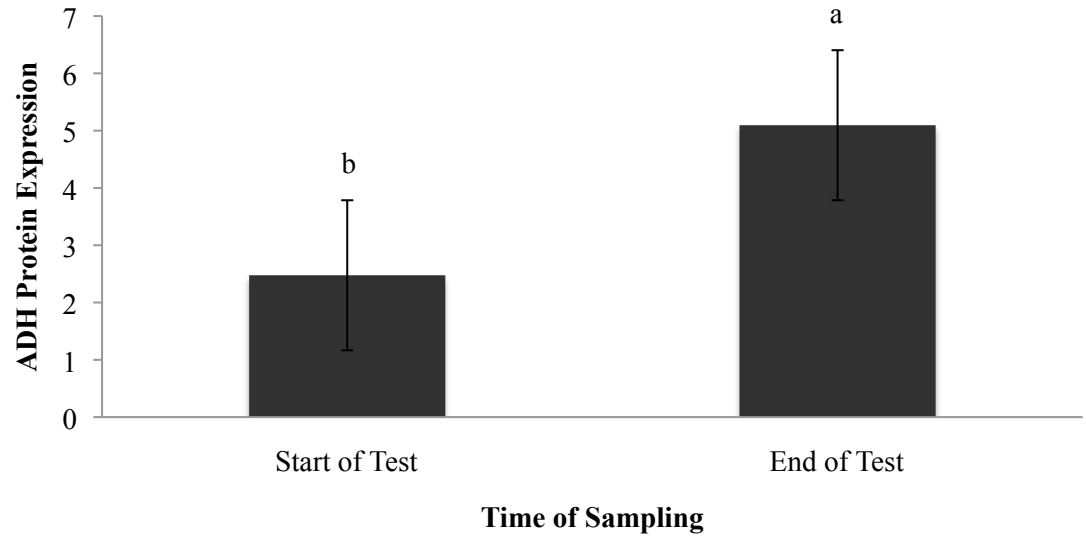

Figure 4.6. Relative expression of ADH1C from hepatic tissue measured at the start and end of the finishing period. Bars with differing superscripts are significantly different (P<0.05). Error bars indicate the SEM.

Alcohol dehydrogenase 1 C levels in hepatic tissue were higher (P=0.0287) at the end of test relative to the start of test (Figure 4.6). It is unclear why this variation in ADH1C enzyme levels occurred over the span of the trial. Under vitamin A restriction, RALDH1 activity is increased 3-fold (Napoli *et al.* 1996). Perhaps the significant reduction in liver retinol at the end of test increased ADH1C activity, and subsequently increased enzyme synthesis. Measurement of RAL and RA levels in adipose tissue should be examined to evaluate if the proposed mechanism is correct.

4.4. Conclusion

Data obtained on IMF deposition and protein quantification in this study indicate that those animals TT at *ADH1C c.-64T>C* should have enhanced IMF deposition in the LT muscle under vitamin A restriction. However, as the majority of steers in the current trial graded Canada AAA, variation between treatments and genotypes was not observed at the 25% and 50% inclusion levels. Therefore, the determination of an optimal vitamin A supplementation level was not possible. The results suggest that the impact of an interaction between vitamin A supplementation level and *ADH1C* genotype on fat deposition may be influenced by the growth stage of the animal. Before this research can be applied to the beef industry, further investigation should look at the impact of finishing length and thus, growth stage, on the interaction between *ADH1C* genotype and vitamin A supplementation level. The potential impact of hormonal implants on the effect of this interaction on carcass traits should also be evaluated.

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APPENDIX A

Feedlot Performance and Carcass Data for 117 steers

Tag		VA	Fin,	HCW	BF	REA	Ultrasound	Grader		
#	Geno	trt	Wt. (kg)	(kg)	(mm)	(cm^2)	Score	Score	% Yield	% IMF
25	CC	0%	690	371	12	91.75	4.6	5.3	56.69	5.49
249	CC	0%	654	340	11	84.90	5.3	5.8	54.21	5.28
66	CC	0%	585	331	7	82.95	6.2	7.2	58.72	6.34
221	CC	0%	602	319	10	79.15	4.85	6.2	54.99	6.16
299	CC	0%	623	337	9	86.49	5.35	5.7	56.59	5.90
32	CC	0%	601	336	10	77.91	5.75	5.6	56.01	8.50
135	CC	0%	622	324	10	83.08	5.4	5.5	56.06	5.52
328	CC	0%	639	358	10	87.25	5.45	7.1	57.33	4.83
352	CC	0%	563	309	9	86.19	6.30	8	57.75	10.47
138	CC	25%	633	333	10	83.70	5.9	4.4	55.54	2.79
325	CC	25%	585	310	12	89.38	5.6	6.1	56.51	6.34
48	CC	25%	592	333	11	88.41	5.8	7.8	57.45	9.22
192	CC	25%	572	295	12	80.87	5	5.1	50.27	6.92
329	CC	25%	616	328	10	85.46	5.2	5.9	55.01	4.70
61	CC	25%	582	327	9	84.07	4.9	5.4	56.24	4.91
379	CC	25%	573	292	11	78.16	5.75	6.2	55.08	6.05
242	CC	25%	570	308	8	93.11	4.7	5.9	60.86	6.66
296	CC	25%	585	304	11	88.31	4.75	5.2	55.80	7.21
166	CC	50%	549	298	10	91.59	5.45	5.3	56.35	4.30
358	CC	50%	565	316	10	86.95	4.9	4.8	56.36	3.98
152	CC	50%	634	338	8	75.96	5.25	6.1	51.62	6.29

186	CC	50%	632	351	11	84.20	5.4	5.5	54.85	6.30
428	CC	50%	559	308	11	85.20	4.55	5.7	51.99	6.23
26	CC	50%	622	330	12	90.84	6.4	5.3	55.16	4.91
264	CC	50%	639	347	7	94.11	5.35	5.3	60.52	3.08
44	CC	50%	650	348	10	95.71	5.8	6.1	57.60	6.52
151	CC	.	.	.	.	.	.	.	.	.
43	CT	0%	679	381	10	90.22	6.2	6.8	58.51	8.17
195	CT	0%	598	337	9	82.35	4.7	7.8	56.38	9.72
320	CT	0%	584	315	9	88.27	5.4	5.4	57.71	5.00
391	CT	0%	611	353	8	92.53	5.6	5	55.01	3.27
76	CT	0%	670	369	11	91.48	5.2	5.6	53.97	5.24
199	CT	0%	591	352	10	100.18	4.6	5.8	64.58	3.24
446	CT	0%	656	360	11	89.68	5.7	6.1	54.89	6.32
89	CT	0%	655	369	12	90.19	7	7.6	53.62	12.82
103	CT	0%	596	328	13	98.75	5.5	5.2	55.05	4.10
218	CT	0%	601	334	8	76.91	5.8	5.7	58.01	7.66
313	CT	0%	569	310	9	82.20	6.15	5.4	56.17	7.00
253	CT	0%	594	314	10	94.40	5.7	5.3	54.58	6.56
268	CT	0%	645	358	13	93.06	5.95	6.1	50.16	7.59
309	CT	0%	583	324	10	91.38	5.15	6.5	58.74	6.75
348	CT	0%	578	318	8	84.24	5.5	5.4	57.33	5.08
64	CT	25%	623	356	14	95.22	5.9	5.6	57.24	9.07
211	CT	25%	579	321	10	83.21	5.2	5.4	52.35	6.05
409	CT	25%	623	340	11	95.36	5.1	5.5	54.66	3.88
7	CT	25%	592	332	10	84.26	5.7	7.4	57.92	5.64
27	CT	25%	732	402	13	94.52	5.8	5.8	49.14	6.47
90	CT	25%	552	300	8	75.74	4.65	4.9	53.34	3.60

412	CT	25%	595	331	11	91.54	5.6	5.7	57.29	6.87
227	CT	25%	621	347	10	86.20	4.6	4.4	56.15	3.69
276	CT	25%	591	307	12	86.25	5.4	5.9	54.59	8.23
317	CT	25%	590	315	10	82.33	5.4	5.9	56.04	6.29
405	CT	25%	573	304	10	69.42	5.1	5.3	51.69	6.91
2	CT	25%	557	305	10	82.88	5.8	7.2	54.20	6.35
16	CT	25%	611	341	10	84.15	4.45	6	56.99	6.05
240	CT	25%	572	303	13	76.84	5.8	5.4	54.04	5.37
303	CT	25%	614	329	14	76.99	4.85	4.4	53.37	4.70
172	CT	50%	631	346	13	75.14	5.2	4.8	50.50	4.94
297	CT	50%	640	338	11	90.35	6	7.7	58.06	8.42
413	CT	50%	615	340	15	92.68	5.7	6.5	51.85	9.06
441	CT	50%	571	305	13	80.31	5.2	6.1	49.80	5.04
20	CT	50%	663	358	11	86.91	5.1	4.8	49.47	4.70
210	CT	50%	610	340	12	81.55	5.4	5.8	54.57	5.50
289	CT	50%	658	367	9	100.15	6	6.2	59.80	5.71
56	CT	50%	637	352	10	90.87	5.1	5.2	56.93	5.28
85	CT	50%	588	327	9	80.50	4.85	5.9	55.08	5.18
202	CT	50%	603	332	11	95.17	6.3	5.6	57.78	5.61
207	CT	50%	586	306	10	72.65	5	4.5	54.57	4.36
59	CT	50%	586	320	7	76.58	4.4	4.8	59.84	4.90
215	CT	50%	573	310	15	87.58	5.7	6.8	53.34	8.64
369	CT	50%	525	281	4	86.68	4.3	4.7	58.59	2.91
425	CT	50%	579	316	13	76.18	5.2	6.5	53.06	4.33
65	TT	0%	573	309	.	.	.	5.8	54.73	7.04
226	TT	0%	604	344	15	98.67	5.85	6.1	52.48	6.23
321	TT	0%	639	358	11	90.39	5	5.7	59.72	4.15

330	TT	0%	589	322	10	86.68	5.8	5.9	55.64	8.91
75	TT	0%	654	369	11	96.34	4.55	4.8	53.51	4.74
104	TT	0%	594	330	9	91.42	5	4.7	59.37	3.15
360	TT	0%	619	341	8	88.81	5.55	5.5	56.82	3.94
9	TT	0%	677	366	7	89.34	5.6	4.7	57.68	5.66
10	TT	0%	569	317	10	75.74	6.2	8.3	53.22	9.09
46	TT	0%	614	347	9	81.31	5.9	5.8	55.42	4.49
87	TT	0%	538	310	10	88.71	4.6	5.2	60.19	4.50
51	TT	0%	648	354	11	83.65	5.75	7.1	57.27	7.09
113	TT	0%	631	337	10	90.18	5	5.5	59.18	5.23
114	TT	0%	541	296	8	79.39	5.6	5.9	59.03	5.34
121	TT	0%	621	344	10	83.61	5.8	7.8	51.34	7.27
191	TT	25%	565	305	7	75.77	4.85	4.8	57.31	2.54
285	TT	25%	572	317	11	90.12	5.85	6.2	52.42	8.03
295	TT	25%	616	342	9	88.54	5.55	6.8	53.70	8.16
439	TT	25%	566	307	11	76.26	5.5	4.8	53.20	5.50
154	TT	25%	622	336	14	84.15	5.85	5.5	51.18	6.16
243	TT	25%	579	333	11	75.88	6.3	8.5	54.70	10.26
337	TT	25%	596	332	10	84.58	4.8	6	57.50	5.55
436	TT	25%	629	345	6	92.07	5	5.1	58.49	3.65
333	TT	25%	560	310	11	88.33	5.8	5.5	53.69	4.83
432	TT	25%	610	327	10	88.81	4.65	5.3	55.60	5.99
443	TT	25%	602	327	14	78.35	5.6	5.1	52.66	5.52
78	TT	25%	624	342	10	96.45	5.4	6.8	56.57	7.39
96	TT	25%	598	319	12	81.61	5.2	4.7	56.94	3.91
241	TT	25%	620	346	11	89.94	6.45	7.9	57.11	7.64
255	TT	25%	559	303	16	91.38	5.15	5.8	56.02	8.34

41	TT	50%	604	335	12	97.38	5.95	5.6	57.66	7.83
77	TT	50%	626	342	14	94.03	6.3	9.1	48.46	14.86
356	TT	50%	608	330	11	94.27	6.2	6.2	56.87	7.66
382	TT	50%	565	318	.	.	.	6.5	50.02	8.27
52	TT	50%	588	323	8	85.55	4.5	5.9	57.16	5.64
206	TT	50%	597	330	10	82.64	6.3	6.5	55.66	9.03
415	TT	50%	599	328	7	84.73	5.8	7.1	59.53	8.28
30	TT	50%	609	323	13	96.90	6.7	8.9	57.71	12.09
47	TT	50%	646	340	10	79.19	4.6	4.7	55.18	3.70
318	TT	50%	569	308	7	95.12	4.9	5.1	60.59	4.80
394	TT	50%	600	341	12	93.35	6.2	5.9	61.39	6.85
120	TT	50%	600	331	15	83.46	6.1	7.7	50.68	10.48
155	TT	50%	581	310	7	88.89	5.45	5.3	61.06	4.85
288	TT	50%	656	352	10	98.84	4.9	4.9	58.52	5.51
384	TT	50%	635	347	10	78.88	5.1	5.4	56.02	5.91

APPENDIX B

Serum and Liver Retinol Data – Intensive Subpopulation (n=45)

Animal #	Genotype	VA trt	SOT Serum (ppm)	EOT Serum (ppm)	SOT Liver (ppm)	EOT Liver (ppm)
25	CC	0%	0.44	0.21	399.6	27.39
66	CC	0%	0.62	0.24	192	6.63
221	CC	0%	0.55	0.15	138.6	0.6
249	CC	0%	0.81	0.28	270	0.63
299	CC	0%	0.68	0.29	253.2	4.65
48	CC	25%	0.46	0.29	279	69
138	CC	25%	0.56	0.21	93.6	3.87
192	CC	25%	0.63	0.19	66.3	0.6
325	CC	25%	0.85	0.26	200.7	0.6
329	CC	25%	0.67	0.36	168.3	1.89
152	CC	50%	0.62	0.31	184.5	11.37
166	CC	50%	0.8	0.34	30	16.08
186	CC	50%	0.62	0.27	138.6	5.19
358	CC	50%	0.8	0.37	320.1	1.92
428	CC	50%	0.63	0.26	177.9	0.69
43	CT	0%	0.51	0.29	265.2	19.89
199	CT	0%	0.7	0.22	32.1	0.6
320	CT	0%	0.67	0.25	234.6	1.77
391	CT	0%	0.61	0.27	55.8	0.69

446	CT	0%	0.64	0.2	173.7	0.6
7	CT	25%	0.7	0.27	471.3	2.91
64	CT	25%	0.64	0.27	129	2.01
90	CT	25%	0.7	0.24	164.1	25.56
211	CT	25%	0.55	0.27	189.6	1.35
412	CT	25%	0.66	0.32	247.2	0.6
20	CT	50%	0.75	0.3	327.3	23.07
172	CT	50%	0.64	0.36	78.6	10.62
210	CT	50%	0.74	0.26	14.7	0.69
297	CT	50%	0.66	0.3	184.2	4.44
441	CT	50%	0.69	0.42	191.1	0.66
65	TT	0%	0.62	0.26	279.9	23.76
75	TT	0%	0.63	0.24	177.3	1.44
104	TT	0%	0.56	0.19	148.2	2.94
226	TT	0%	0.61	0.26	135.9	0.6
360	TT	0%	0.68	0.26	243	0.6
191	TT	25%	0.51	0.26	.	0.87
285	TT	25%	0.54	0.23	183.3	5.07
295	TT	25%	0.6	0.22	154.8	13.47
337	TT	25%	0.6	0.29	400.2	9.9
439	TT	25%	0.65	0.27	116.7	0.6
52	TT	50%	0.55	0.34	288.9	2.46
77	TT	50%	0.79	0.26	160.8	9
206	TT	50%	0.66	0.21	52.2	0.6
356	TT	50%	0.74	0.35	59.4	0.6

415	TT	50%	0.7	0.3	103.8	0.6

APPENDIX C

Table 5 Composition and nutrient analysis of backgrounding and finishing rations on a dry-matter basis.

	Backgrounding	Finishing
Diet Composition, % DM		
Barley Silage	9.71	2.22
Grass Hay	15.70	-
Barley Grain	44.96	82.36
Canola Meal	-	5.26
Wheat DDGS	9.2	-
Barley Straw	14.05	4.83
Supplement[1]	6.39	5.32
Supplement Composition[1]		
Barley	745.2	481.2
Limestone	184.8	250
Salt	42.2	205
Mono-calcium Phosphate	11.4	42.2
Canola Oil	10	15
Vitamin E	1	1
Manganese Oxide	0.75	0.78
Zinc Oxide	0.75	0.75
Copper Sulfate	0.73	0.67
Sodium Selenite	0.67	0.24
EDDI	0.02	0.02
Vitamin D	0.02	0.01
Cobalt Carbonate	0.01	0.01
Rumensin	2.4	3.1
Nutrient Analysis, % DM		
TDN	65.35	75.06
CP	12.3	13.42
ADF	23.05	10.08
NDF	37.6	22.1
Ca	0.71	0.64
P	0.36	0.40
Vitamin A (IU/kg DM)[2]	3664	555

[1]kg DM/1000 kg supplement.
[2]Calculated as 400 IU/mg B-carotene (NRC 1970).

APPENDIX D

Plant and Animal Genome XXII Conference, January 10-15, 2014, San Diego, CA.

Effect of varying dietary vitamin A supplementation levels in combination with ADH1C genotype on intramuscular fat deposition in finishing beef steers

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Previously, *ADH1Cc.-64T>C* was shown to have an association with intramuscular fat (IMF) in the *longissimus dorsi* (LD) muscle when vitamin A was limited in finishing rations. The purpose of the current study was to determine the optimum vitamin A supplementation level, in combination with *ADH1C* genotype, to increase IMF of the LD muscle. Forty-five TT, 45 CT and 27 CC Black Angus-cross steers were backgrounded on a commercial ration containing 3360 IU vitamin A/kg DM. During finishing the steers were randomly assigned to one of three vitamin A treatments at 25, 50 and 75% of the NRC recommendation of 2200 IU/kg DM. Treatments were administered via an oral bolus. Carcass quality was evaluated and a sample from the LD muscle was collected for analysis of IMF. A treatment x genotype interaction (P=0.038) was observed for IMF; TT steers on the 75% treatment had higher IMF relative to CT and CC steers on the same treatment. Intramuscular fat was also higher for TT steers on the 75% treatment in comparison to TT steers on the 25% treatment. Eighty-four percent of the steers graded USDA AAA. Western blot analysis showed that TT steers had higher (P=0.0176) ADH1C levels in liver. Previously, TT steers had increased IMF when fed limited vitamin A. In the current study the lack of variation between treatments and genotypes was likely due to the majority of the steers grading USDA AAA. However, the western blot data shows TT steers are expected to have higher IMF deposition.